IJPT
1735-2657/03/21-15-17
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 2:15-17, 2003

Stereospecific Determination of Mefloquine in Whole Blood by HPLC

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Received January 12, 2003; Revised February 15, 2003; Accepted February 30, 2003

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

Mefloquine, as a racemic mixture, is used for the treatment and prophylaxis of malaria. Stereoselective pharmacodynamic and pharmacokinetic differences has been observed for mefloquine. In this study a modified stereoselective HPLC method is presented for determination of mefloquine (MFQ) enantiomers in whole blood. The assay involved liquid-liquid extraction of MFQ from biological fluids with methyl tert-butyl ether in the presence of sodium hydroxide and derivatization of the residue by (+)-1-(9-fluorenyl) ethyl chloroformate (FLEC) as chiral derivatizing reagent. Separation of the resulting diastereomers was performed on a Novapack C18 reversed-phase cartridge column using acetonitrile, water, glacial acetic acid (730:270:0.7, v/v/v) as the mobile phase with a flow-rate of 1 mL/min. Using 500 µL of whole blood, the limit of determination was 50 ng/mL with fluorescence detection with excitation at 263 nm and emission at 475 nm for both enantiomers. This method is comparatively simple and practical for the determination of small amounts of mefloquine enantiomers.

Keywords: *Mefloquine, Enantiomer, Derivatization, Whole blood, RP-HPLC*

Mefloquine (MFQ), *rac*-erythro-α-(2-piperidyl)-2, 8 -bis(trifluoromethyl)-4-quinolinemethanol (Fig 1), has been used for the treatment and prophylaxis of malaria as a racemic mixture. Conflicting results on the antimalarial activity of MFQ enantiomers have been reported. In some reports, no significant difference was observed between antimalarial activities of enantiomers against *Plasmodium berghei* or *Plasmodium yoelli* in rodents [1, 2] and human Plasmodium falciparum in vitro [3]. In other reports the (+)-(RS)-enantiomer of MFQ was more active than the (-)-(SR)-enantiomer against different strains of *Plasmodium falciparum* [4]. Higher concentrations of (-)-(SR)-MFQ than (+)-(RS)-enantiomer in human plasma and blood are also reported [5-7].

Different direct or indirect methods have been reported previously for determination of mefloquine enantiomers [5, 8-12]. An indirect HPLC method using (-)-1-(9-fluorenyl) ethyl chloroformate as derivatizing reagent is reported by Bergqvist et al [11].

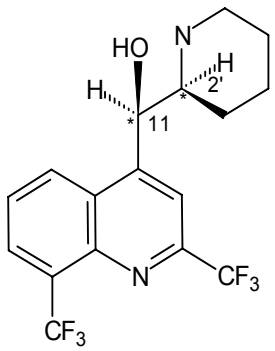
In the present study (+)-1-(9-fluorenyl) ethyl chloroformate is used for the preparation of MFQ diastereomers followed by a modified achiral reversed-phase HPLC technique using fluorescence detection partially based on the previously reported procedure [11].

MATERIALS AND METHODS

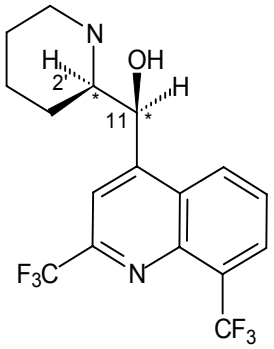
Chemicals and reagents. Racemic MFQ-HCl was purchased from Roche (Basel, Switzerland). The (-)-(SR)- and (+)-(RS)-MFQ enantiomers were resolved with (+)-3-bromo-8-camphorsulphonic acid ammonium salt (Aldrich, Milwaukee, WI, USA) according to Carrol and Blackwell [1]. The derivatizing reagent, (+)-1-(9-fluorenyl) ethyl chloroformate (FLEC) (18 mM in acetone) was purchased from Fluka (Buchs, Switzerland). The optical purity of the reagent was higher than 99.5%. All other chemicals and solvents were of either chromatographic or analytical reagent grade from Merck (Darmstadt, Germany).

Standard solutions. A stock solution of *rac*-MFQ was prepared by dissolving 43.85 mg MFQ-HCl in 10 mL methanol to a final concentration of 4 mg/mL. Standard solutions were prepared by subsequent dilution of the stock solution with methanol. All solutions were stored at +4°C.

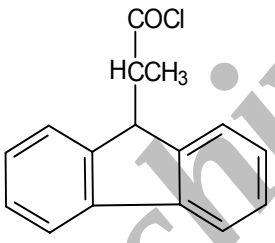
A solution of 36 µM (+)-FLEC was prepared by diluting 200 µL 18 mM (+)-FLEC in acetonitrile. The reagent solution was stored at -20°C until used.



(+)-Mefloquine 11R, 2'S



(-)-Mefloquine 11S, 2'R



rac-9-(fluorenyl) ethyl chloroforma

Fig 1. Chemical structure of mefloquine (MFQ) and derivatizing reagent.

Borate buffer (43 mM) was prepared by dissolving 0.26 g boric acid and 0.32 g potassium chloride in approximately 80 mL distilled water, adjusted to pH 8.5 with 1M sodium hydroxide solution and made to 100 mL volume with water.

Chromatographic conditions. The chromatographic system consisted of a 510 HPLC pump, a WISP 717 autoinjector, a 486 fluorescence detector and a 476 integrator (Waters Milford, MA, USA).

The reversed-phase system consisted of a Novapak C18 (150 mm x 3.9 mm, 5 µm) cartridge column (Waters, Milford, MA) and a mobile phase of acetonitrile-water-glacial acetic acid (730:270:0.7, v/v/v) at a flow rate of 1 mL/min. Fluorescence detection was performed using a detector with excitation at 263 nm and emission at 475 nm.

Sample preparation. Frozen blood samples were thawed and allowed to reach room temperature. A 500 µL aliquot of blood was placed into a siliconized glass test tube, 50 µL of standard solution of mefloquine was added, mixed, capped and left at room temperature for 20 min. 500 µL of sodium hydroxide 0.2 N and 5 mL of methyl tert-butyl ether were added successively. The test tube was vortex-mixed for 30 s and centrifuged at 1000 g for 10 min. The organic layer was transferred to a clean siliconized test tube and evaporated to dryness at 60°C under nitrogen.

Derivatization. The extraction residue was reconstituted in 100 µL of 36 µM (+)-FLEC and 50 µL (43 mM) borate buffer. The mixture was vortexed and allowed to react at room temperature for 40 min. After centrifugation at 1000 g for 10 min, 20 µL of the solution were loaded into the HPLC column.

Calibration, accuracy and precision. Calibration curves were prepared by spiking (500 µL blank human blood) with 50 µL of MFQ standard solution to give final concentrations in the range of 50-2000 ng/mL. The sample extraction, derivatization and HPLC analysis was performed as described above. Calibration curves were constructed by plotting the measured peak area of each MFQ diastereomer versus concentrations of each enantiomer of MFQ in blood.

The accuracy and precision of the method were calculated by determination of three replicate samples of MFQ at concentrations of 50, 100, 500 and 2000 ng/mL of each enantiomer on three separate days.

RESULTS AND DISCUSSION

The proposed HPLC technique is based on the method reported by Bergqvist et al [11] with some modifications. Derivatization of MFQ enatiomers was performed using (+)-FLEC instead of (-)-FLEC. Various columns and different combinations of mobile

Table 1. Accuracy and precision in spiked blood (n= 9; three sets for three days)

Concentration added (ng/mL)	(-)-(SR)-Mefloquine			(+)-(RS)-Mefloquine		
	Calculated (mean±S.D.)	%C.V.	%Error	Calculated (mean±S.D.)	%C.V.	%Error
Intra-day (n=3)						
50	49.6 ± 5.8	11.6	-0.8	51.9 ± 5.2	10.0	3.8
100	100.2 ± 5.4	5.4	0.2	103.6 ± 5.9	5.7	3.6
500	508.4± 22.9	4.5	1.7	523.3 ± 35.7	6.8	4.7
2000	1976.6 ± 58.4	2.9	-1.2	1969.2 ± 77.3	3.9	-1.5
Inter-day (n=9)						
50	51.6 ± 7.0	13.5	3.2	52.3 ± 6.7	12.8	4.6
100	100.5 ± 4.7	4.7	0.5	101.3 ± 6.5	6.4	1.3
500	505.1 ± 31.9	6.3	1.0	512.8 ± 29.2	5.7	2.6
2000	1999.6 ± 47.1	2.4	-0.0	1985.2± 83.1	4.2	-0.7

phases were tested to find the optimum condition of chromatography. Best result was obtained by using Novapack C18 cartridge column and a mixture of acetonitrile, water and acetic acid (730:270:0.7, v/v/v) as mobile phase. Good baseline resolution of both enantiomers was achieved (α >1.3) without any interfering peak. In this system two unequal peaks of (-)-(SR)- and (+)-(RS)-MFQ was observed with retention times of about 10 and 12.7 min (Fig 2). The elution order of the diastereomers was identified using pure enantiomers. The fluorescence response of the (-)-(SR)-MFQ diastereomer was about 4.5 times higher than the (+)-(RS)-MFQ diastereomer.

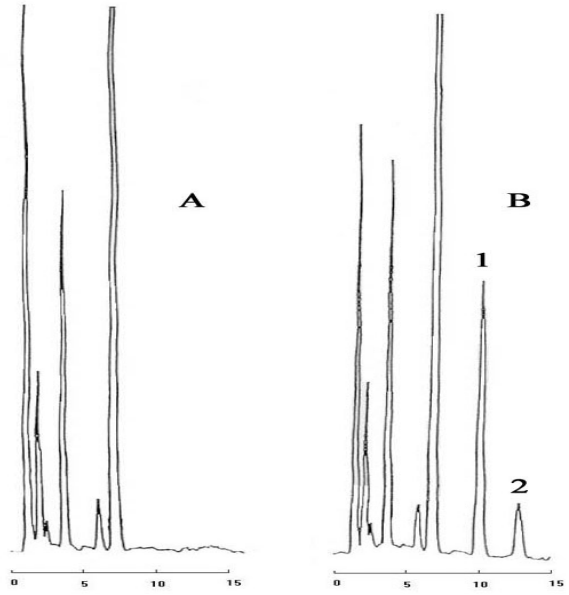


Fig 2. HPLC chromatograms of MFQ diastereomers. (A) Drug free blood; (B) Blood sample spiked with MFQ enantiomers (1 µg/ml of each enantiomer). Peaks: 1, (-)-(SR)-MFQ diastereomer; 2, (+)-(RS)-MFQ diastereomer.

Optimal conditions of derivatization reaction were selected by studying the influence of (+)-FLEC concentration, borate buffer concentration, the reaction time and the temperature. The best result was achieved with 43 mM borate buffer and 36 µM (+)-FLEC as derivatizing reagent. The reagent concentration was about 1/10 of the reported concentration for (-)-FLEC. The reaction was allowed to proceed at different time intervals at room temperature. The maximum peak intensity of the diastereomers was observed after 40 min which is consistent with that reported by Bergqvist et al [11]. The standard calibration curves constructed by plotting peak areas of MFQ diastereomers against the concentrations of each enantiomer exhibited good linearity (r^2 > 0.98). Typical equations describing the linearity were Y=278.7 X-3158.6 for (-)-(SR)-MFQ and Y=74.3 X+734.1 for (+)-(RS)-MFQ over the range of 50-2000 ng/mL.

The accuracy and precision data for the determination of MFQ enantiomers in whole blood are presented in Table 1. The data shown in this table is in support of validation of the proposed method. The limit of determination of each enantiomer was 50 ng/mL with a C.V. better than 13.5% using 500 µL of whole blood.

CONCLUSION

A modified indirect method for the determination of MFQ enantiomers in blood is reported using (+)-FLEC as derivatizing reagent. Based on a commercially available reagent and fluorescence detection, this method is practical and suitable for determination of small amounts of MFQ enantiomers for pharmacokinetic studies.

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